

**CLAIMS**

1. A method for isolating minor components such as squalene, carotenes, tocots and sterols from a vegetable oil under an isocratic condition, said method comprising the steps of:

- 5        a) esterification of the oil with an alcohol, said esterification provides a mixture of a glycerol, fatty acids esters and the minor components,
- b) collection of the esters phase containing the minor components from the glycerol;
- c) distillation of the esters phase, said distillation provides a concentrate of squalene, carotenes, tocots and sterols at a temperature from room temperature to 200°C and under a pressure from 0 to 150 mm Torr;
- 10        d) dilution of the concentrate in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar;
- 15        e) adsorption of the concentrate obtained from step (d) on an adsorbent;
- f) extraction of the minor components from the concentrate obtained from step (e) by desorption of the minor components using a predetermined mixture of solvents which is a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar; and
- g) desorption of the minor components by a predetermined mixture of solvents.

2. A method as claimed in claim 1, wherein the vegetable oil is selected from palm oil.

3. A method as claimed in claim 1, wherein the alcohol in step (a) is selected from lower alcohols such as methanol, ethanol, isopropanol and butanol.

4. A method as claimed in claim 1, wherein the adsorbents used in step (e) is selected  
5 from normal-phase silica gel, reversed-phase (particularly C18) silica gel or neutral alumina and polymer adsorbents such as polyethylene glycol and polyacrylate polyalcohol.

10 5. A method as claimed in claim 1, wherein the solvent is selected from non-polar solvents such as hexane, heptane, dichloromethane, cyclohexane, petroleum ether, ethyl acetate, isoctane or cyclohexane.

15 6. A method as claimed in claim 1, wherein the solvent is selected from polar solvents such as ethanol, isopropanol, methanol, butanol or acetonitrile.

20 7. A method for isolating individual carotene such as  $\beta$ -carotene,  $\alpha$ -carotene, lycopene, phytoene and phytofluene, individual tocol such as  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocopherol and tocotrienol and individual sterol such as  $\beta$ -sitosterol from vegetable oil under isocratic and isobaric conditions under a pressure between 0.2 – 1000 bar, the method comprising the steps of:

- a) adsorbing the oil to an adsorbent and
- b) desorbing the mixture obtained in step (a) a mixture of polar and non-polar solvents.

8. A method as claimed in claim 7, wherein the source of the individual carotene is concentrate or phytonutrients rich-fractions.

9. A method as claimed in claim 7, wherein the vegetable oil is selected from palm oil.

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10. A method as claimed in claim 7, wherein the adsorbent is selected from normal-phase silica gel, reversed-phase silica gel or neutral alumina, preferably reversed-phase C18 silica gel and polymer adsorbents such as polyethylene glycol and polyacrylate polyalcohol.

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11. A method as claimed in claim 7, wherein the non-polar solvent is selected from hexane, heptane, ethyl acetate, isoctane or cyclohexane.

12. A method as claimed in claim 7, wherein the polar solvent is selected from ethanol, isopropanol, methanol or butanol.

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13. A method for isolating individual carotene such as  $\beta$ -carotene,  $\alpha$ -carotene, lycopene, phytoene and phytofluene, individual tocol such as  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocopherol and tocotrienol and individual sterol such as  $\beta$ -sitosterol from minor components such as squalene, carotenes, tocols and sterols obtained from a method which is conducted under an isocratic condition, the method comprising the steps of:

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a) esterification of the oil with an alcohol, said esterification provides a mixture of glycerol, fatty acids esters and the minor components,

- b) collection of the esters phase containing the minor components from the glycerol;
- c) distillation of the esters phase, said distillation provides a concentrate of squalene, carotenes, tocols and sterols at a temperature from room temperature to 200°C and under a pressure from 0 to 150 mm Torr;
- d) dilution of the concentrate in a non-polar solvent or a mixture of non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar;
- e) adsorption of the concentrate obtained from step (c) on an adsorbent;
- f) extraction of the minor components from the concentrate obtained from step (d) by desorption of the minor components by a predetermined mixture of solvents which is a non-polar solvent or a mixture of a non-polar solvent and a polar solvent with a ratio ranges from 90:10 to 99.5:0.5 with a pressure from 0.2 to 50 bar; and
- g) desorption of the minor components by a predetermined mixture of solvents; characterised in that the minor components obtained from the above method undergo a further extraction under isocratic and isobaric conditions under a pressure between 0.2 – 1000 bar, the extraction comprising the steps of:
  - 1) adsorbing the minor components to an adsorbent and
  - 2) desorbing the mixture obtained in step (a) a mixture of polar and non-polar solvents.

14. A method as claimed in claim 13, wherein the vegetable oil used in step (a) is selected from palm oil.

15. A method as claimed in claim 13, wherein the adsorbent for step (1) is selected from 5 normal-phase silica gel, reversed-phase silica gel or neutral alumina, preferably reversed-phase C18 silica gel and polymer adsorbents such as polyethylene glycol and polyacrylate polyalcohol.

16. A method as claimed in claim 13, wherein the non-polar solvent for step (2) is 10 selected from hexane, heptane, ethyl acetate, isoctane or petroleum ether.

17. A method as claimed in claim 13, wherein the polar solvent for step (2) is selected from ethanol, isopropanol, methanol, butanol ethyl acetate or acetonitrile.